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LETTER TO THE EDITOR

EVALUATION OF THE "SYMBIOSYS" IMMUNOASSAY FOR THE SEROLOGICAL DIAGNOSIS OF CHAGAS DISEASE

São Paulo, December 6, 2011

Dear Sir,

For the laboratory diagnosis of Chagas disease several tests are commonly used. Sensitivity and specificity must always be taken into account, as well as the fact that these methods vary when we are dealing with an acute or chronic stage of the parasitosis[3]. Also, it is important to remember that the initial stage of *Trypanosoma cruzi* infection is characterized by the presence of the parasite in the blood and not by clinical or epidemiologic findings, for example. As for the diagnosis in the chronic stage, serological tests are usually the first choice.

In this context, new procedures are commonly proposed as new diagnosing tools, but it should be noted that they need to show further advances, especially in regard to sensitivity, specificity, ease of implementation, availability and cost.

To carry out the serological studies exemplifying these interests we mention the use of purified, recombinant, synthetic or excretory-secretory antigens with different strains of *T. cruzi*[1,4,5,6,7,8]. Some results were important and significant in attempts to show improvements in this new technique.

In the laboratories where we provide care and research activities, we often analyze the merits of submitted techniques for the purpose of providing assistance (Laboratório de Parasitologia do Instituto de Medicina Tropical da Universidade de São Paulo; Laboratório de Investigação Médica-Parasitologia do Hospital das Clínicas, da Faculdade de Medicina da Universidade de São Paulo). Now, with the recent development of ELISA Anti- Chagas Sym (Symbiosys) enzyme immunoassay, which will probably soon be commercialized, and keeping a conduct adopted by us several times in the past, we feel it would be advisable to know the effectiveness of it in comparison with the Chagas Elisa III - Ebram[2], widely used by us and, therefore, with which we are familiar.

The Anti-Chagas Sym immunoenzymatic assay (Symbiosys Diagnóstica Ltda., Lot 1016500010, used before the expiration date) uses microplates with wells that are coated with recombinant *T. cruzi* antigen, in which we place the samples. During incubation the antibodies possibly present in the samples tend to form a complex with the antigens of the microplate wells.

After aspiration and wash, all other components that are unlinked with the antigens are removed. In a second incubation the antigen-antibody complex is detected by the addition of a conjugate (anti-IgG antibodies conjugated with radish peroxidase) and the components that are connected in a non-specific manner are removed during the washing of the plate.

The enzyme activity fixed in the solid phase, acting with the chromogen solution generates an optical signal that is proportional to the amount of anti-*Trypanosoma cruzi* antibodies in the sample. The intensity of color is measured by spectrophotometer reading at 450 nm with a reference filter of 620/630.

Radish peroxidase is an enzyme isolated from wild radish that is capable of acting as antigens, also being used as a histochemical marker in optical and electronic microscopy. Its antigenicity allowed its use as an antigen marker in experimental immunology.

The Chagas Elisa III - Ebram immunoassay[2] (EBRAM - Produtos Laboratoriais Ltda. Lot 02519A0827 used before the expiration date) uses microplates with wells coated with total extracts of *T. cruzi, Tulahuen* and *Mn* strains, including membrane antigens that are highly immunogenic. The wells' treatment process is achieved through the use of new technology. A biological adhesive facilitates the immobilization of antigens thus increasing the plate stability. If the samples contain *T. cruzi* antibodies, there will be formation of a stable complex with the antigens that coat the wells.

The material attached in a non-specific manner will be removed by washing. During incubation with the conjugate, the human anti-IgG antibodies labeled with peroxidase will adhere to the formed complex. Finally, after incubation with the chromogenic substrate, the peroxidase attached to the complex will produce an optical sign that will allow us to select the samples that are *T. cruzi* positive. The enzymatic reaction will be ended with the addition of sulfuric acid. Then, the measurement of the color intensity at 450 nm in a colorimetric plate reader (ELISA Bio-Rad, model 2100) for both procedures were made and compared. The cut-off value is determined by multiplying the sum of the averages of the positive and negative controls by 0.22 (Sym) or 0.35 (Ebram), and the gray zone result is determined from the subtraction and addition of 10% of the cut-off for the two tests.

We used 158 serum samples from patients under care in the Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo aiming to establish a possible diagnosis of Chagas disease. We received only the medical requests, without any further information about the nature of the

cases. We found by both methods, 57 positive cases and 101 negative cases. Therefore, there was full agreement (100%), showing identical sensitivities and reliability, at least according to this assessment. We did not notice any operational difficulties. The cost is unknown to us.

In conclusion, we evaluated the sensitivity of the ELISA Anti-Chagas Sym (Symbiosys), a new tool for the serological diagnosis of Chagas disease that will probably be commercialized soon. This procedure uses *Trypanosoma cruzi* recombinant antigens and anti-IgG antibodies conjugated with radish peroxidase, with participation of a substrate-chromogen solution that generates an optical signal. For comparison we used the Chagas Elisa III-Ebram[2], an already well known test which depends on total extracts of *T. cruzi* strains and membrane antigens that are highly immunogenic. In the comparison between the two tests we examined 158 sera to identify any that might be affected by Chagas disease. Fifty-seven tests were positive and 101 were negative, with a complete conformity between the two methods under consideration. We did not notice any operational difficulties in the 90 minutes it took to run the test. The ELISA Anti-Chagas Sym (Symbiosys) showed acceptable sensitivity.

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